WHO Informal Consultation: Development of Viral Vectored Vaccines for HIV, Malaria, Tuberculosis and other Indications

Geneva, 1-2 October 2013

<u>**Overview**</u>

Novel vaccines for the prevention or treatment of infectious diseases such as HIV, HCV, malaria, TB as well as various oncology indications are a major unmet need. Pre-clinical and clinical evidence supports the role of T cell immunity and in particular CD8+ T-cells in the clearance of intracellular pathogens and tumour cells. Efficient induction of CD8+ T-cell responses against a given antigen follows intracellular delivery of the gene encoding for the antigen along with suitable pathogen-derived innate activators, thereby mimicking the native pathway of antigen processing and MHC class I presentation. The use of replication deficient viruses as antigen delivery vectors has been shown to be a safe and robust mechanism to induce both effector cell mediated and humoral immunity, and priming with these vectors can improve magnitude, quality and durability of those responses.

In the ten years since the WHO last convened a meeting of experts to consider the development, production and quality control of viral-vectored vaccine candidates for development, the field has progressed greatly. The lead candidate vaccines in HIV and tuberculosis include viral vectored vaccines, and such vectors also form a major part of the malaria vaccine pipeline. Other pathogens with active viral vectored vaccine approaches under evaluation include dengue virus, influenza virus, ebola virus, hepatitis C virus, respiratory syncytial virus and many others. The list of recombinant viral vectors in pre-clinical and clinical development has expanded and many thousands of subjects have been enrolled in Phase II and Phase III clinical trial programmes in multiple disease areas with recombinant adenovirus and pox viruses being the most advanced platforms. Given the large number of candidates now in clinical studies, there is a need for guidance for vaccine manufacturers to identify appropriate regulatory pathways, development gaps and critical data sets to support the advancement of viral vector based vaccines to licensure. Key leaders from academia, biotech and industry together with clinical scientists and regulatory agencies from the EU, USA and Canada were convened with the objective to review progress in developing viral vectored vaccines and related regulatory considerations.

Since the last WHO meeting, one viral vector based vaccine has been licensed. Sanofi's IMOJEV®, a vaccine against Japanese encephalitis, is based on the yellow fever virus vector 17D, and was recently licensed in Australia [1] based on the review of clinical studies involving over 4,000 children and adults which demonstrated its safety and ability to generate a protective immune response with one dose. However, there have also been disappointing and confounding setbacks, most notably the STEP HIV efficacy trial utilising adenovirus 5 (Ad5) as the vaccine vector, in which the incidence of HIV was shown to be higher in the vaccine group than in the control cohort. This result has caused developers and funders alike to consider the safety and financial risk of further development of any Ad5-based candidate for any vaccine or indication, despite the encouraging malaria human challenge study in which a DNA prime/Ad5 boost regimen was shown to sterilely protect 27% of subjects. Whilst the field continues to examine the STEP trial and other Ad5 clinical data, including the potential detrimental effect of broad pre-existing immunity to this vector, several groups are focused towards identifying alternative human adenovirus serotypes as well as non-human adenoviruses that are equally immunogenic, but with low seroprevalence and that are likely to be perceived as safer candidates. Okairos have a chimpanzee-derived adenovirus (ChAd3) in Phase II for

HCV and have completed 21 human clinical studies, and GenVec have identified a prototype gorilla derived adenovirus vector candidate.

Crucell, Harvard University, the NIH Vaccine Research Center and others are evaluating human Ad35 and Ad26 in the clinic for tuberculosis and HIV, and have demonstrated safety and promising immunogenicity, particularly when these vectors are used in heterologous prime / boost regimens with MVA. The various adenovirus vectors have been shown to differ with respect to their sub-type, seroprevalence, cell-binding receptors, tropism, etc, and induce different gene expression profiles and T-cell and antibody phenotypic functionality, providing the justification to continue to evaluate this class of vectors going forward.

Sanofi's ALVAC-based HIV candidate demonstrated 31% efficacy in a heterologous prime / boost regimen with a recombinant protein adsorbed to alum, and they are optimising the approach in preparation for Phase III evaluation. As clinical candidates advance, developers are contemplating scalability and cost of goods of their production processes, resulting in a drive towards cell line based manufacturing technology. In addition to the regulatory requirements for characterization of the vector, regulators are now needing to consider how to evaluate and guide licensure of a vaccine that contains two different products to support prime / boost, at least one of which is likely to be manufactured utilising a novel continuous cell line.

Status of adenovirus vaccine development

Adenoviral vectors are small, non-enveloped viruses with a double-stranded DNA genome, these viruses are composed of many different serotypes. There are currently human, chimpanzee and gorilla-derived adenoviral vectors in various stages of vaccine development. Human-derived adenovirus 5 (Ad5), Ad26 and Ad35 as well as chimpanzee-derived ChAd3 and ChAd63 are currently in the clinic. Inherent differences between recombinant Ad vectors are based on different adenoviral serotypes used for the vector, differences in vector potency, differences in the TLR ligands presented to the innate immune system and differences in vector construction.

This WHO meeting was held the week following a US National Institutes of Health sponsored gathering to review the combined data of the three HIV efficacy studies that included Ad5 in their regimens, namely the Merck STEP trial, the Merck Phambili study and the NIH Vaccine Research Center (VRC) HVTN 505 study. (For a videolink to this meeting go to: http://www.totalwebcasting.com/view/?id=niaid). The NIH mini-summit meeting sought to address the issues now facing development of HIV and other vaccines that include Ad5 components. Due to the recent occurrence of the NIH mini-summit, several discussions at this WHO meeting reflected on the discussions at the NIH meeting.

Both the STEP and Phambili studies included three doses of three mixed Ad5 vectors each expressing *gag, pol* and *nef* antigens administered at 0, 1 and 6 mo. Following an interim analysis of the STEP trial, an increase in the number of HIV cases in the vaccine group was seen in comparison with the placebo, and the majority of these cases occurred in uncircumcised men with pre-existing Ad5 immunity. On the basis of these data, the more recent HVTN 505 Phase IIb study was designed to enrol Ad5 seronegative volunteers and circumcised men. The HVTN 505 vaccine construct included genes encoding HIV *Env* antigens, and the regimen consisted of three shots of a DNA vaccine expressing *gag, pol, nef, EnvA, EnvB* and *EnvC* at 0, 1 and 2 mo followed by one dose of Ad5 expressing *gag, pol, EnvA, EnvB* and *EnvC* at 6mo. A meta-analysis across the three studies had been performed and was presented to address the following:

1. To evaluate the effect of Ad5 vectored vaccines on HIV infection over time, pooled over the 3 trials

2. To evaluate whether and how the vaccine effect differs for the Merck and VRC vaccines

3. To evaluate whether and how the vaccine effect differs over time period and across baseline subgroups (gender, Ad5 serostatus, circumcision status)

In summary, although pooling of the data across all trials showed an elevated infection rate (33%) in the vaccine group vs. placebo, this risk was driven by the STEP and Phambili studies; the VRC HVTN 505 study alone did not show evidence that the Ad5 vaccine enhanced HIV risk but neither did the study demonstrate efficacy in reducing HIV. The reasons for the differences in the results from the STEP/Phambili and HVTN 505 were the subject of debate and hypotheses include differences in antigen composition (VRC Ad5 vaccine includes *env,* and *gag-pol* fusion rather than individual *gag, pol,* and *nef* genes), different Ad5 backbone (VRC vaccine uses GenVec backbone – E4 and partial E3 deletions in addition to E1, Merck vaccine has deletions in E1), different regimens (DNA priming, one Ad dose in VRC vaccine vs up to three Ad5 doses in Step/Phambili). Analysis is confounded by early unblinding of the Merck Phambili study resulting in most subjects only receiving one of three doses, and potential changes in behavior in the follow up period.

Human adenovirus 5 (Ad5) is seen by some as the gold standard for induction of CD8 T-cell immunity. However, its development has been hampered by the effect of pre-existing immunity on its potency, and safety concerns from the Merck STEP and Phambili studies in which vaccinees experienced increased risk of HIV infection. For this reason there is significant effort to identify alternative low sero-prevalent adenoviral vectors that are comparably potent. There was no explicit attempt to come to consensus at the WHO meeting on the scientific basis for ongoing use of Ad5 vectors, although many at the meeting expressed a view that continuing studies with Ad5 vectors outside the area of HIV vaccines could have a role in experimental medicine, providing the informed consent procedures were thorough, and only very low HIV risk populations were used with information about the potential increased risk of HIV acquisition explicitly discussed.

Chimpanzee adenovirus vectors (Dr. Alfredo Nicosia, Okairos)

In light of the promising, but challenging product development issues associated with human adenovirus-based viral vectors, Okairos is developing a novel platform of replication-defective Ad vectors isolated from chimpanzees (ChAd). Samples have been isolated and screened from over 700 chimpanzees that have been captively bred in the EU and US and more than 100 strains of ChAd have been identified and grouped into 25 distinct serotypes. The immunogenicity of ChAd vectors such as ChAd3 have been shown to be comparable with the most immunogenic adenoviral vectors such as Ad5 in mice and non-human primates, and this potency correlates with the level of antigen expression in dendritic cells. In collaboration with Bob Seder at the NIH, Okairos has shown that the immunological potency is modulated by a vector induced innate response, and there appears to be a balance with antigen expression, i.e. weakly potent vectors such as Ad35 induce strong innate immune responses but low and transient antigen expression, whereas vectors with high potency, such as Ad5 and ChAd3 induce weak innate immune responses but high and long-lasting antigen expression.

Immunological potency, specifically the ability to induce IFNγ, correlates with efficacy as demonstrated by a single injection of ChAd3 in an Ebola virus non-human primate (NHP) challenge model. In this study, ChAd3 was shown to protect challenged animals, whereas all animals had expired in the Ad26 and Ad35 comparator groups. ChAd have also been shown to induce robust

neutralizing antibody responses as demonstrated in preclinical models with RSV infection, and a hepatitis C Phase I vaccine study has shown that responses to ChAd vectors are not hampered by pre-existing Ad5 immunity in humans, and that poly-functional T-cells are induced.

Okairos has also investigated the immunogenicity of ChAd in heterologous prime / boost regimens. In a phase I HCV study in which ChAd3 and hAd6 were evaluated, the immune response was better when the ChAd3 was used as the prime. However, hAd6 boosting was not as efficient as that observed in a preclinical NHP model. There was some correlation with anti-vector neutralising antibodies but none with anti-vector T-cell responses. Prime / boost with MVA, where MVA is used as the boost, has been shown to elicit the highest T-cell responses, consistent with those observed with NHPs, where this regimen induced high levels of memory responses, good antibody responses, as well as different CD8 phenotypes.

Re-administration with the same vector has been shown to boost T-cell responses after several weeks; in fact data suggest that an intervening injection of a heterologous vector (another Ad or MVA) between two administrations of the same ChAd vector improves efficacy of re-administration by establishing a larger pool of antigen-specific memory T cells. Okairos is also investigating the 'mixed route' approach to circumvent the induction of anti-vector antibodies, where the first vector is administered intranasally to prime humoral and cell-mediated responses against the encoded antigen, followed by an intramuscular boost of the same vector.

To date, 21 studies have been completed or are ongoing and 740 subjects have been vaccinated with ChAd. No safety issues have been identified, and the first Phase II prophylactic efficacy study for HCV in high risk individuals is currently ongoing in the US (collaboration with NIH, Johns Hopkins and UCSF)

A Critical Look at Alternative Serotype Adenovirus Vectors for HIV-1 vaccines (Dr. Dan Barouch, Harvard University)

To date and over the last 30 years, there have been four HIV vaccine proof of concept studies in humans: the VaxGen 'AIDSVAX' study in 2003, the Merck 'STEP' study in 2007, the Sanofi RV144 study in 2009 and the NIH VRC HVTN 505 study in 2013. Only the RV144 study has demonstrated efficacy with 31% reduction in HIV acquisition but with no effect on viral load.

In contemplating the questions posed at the NIH mini-summit, Dr Barouch summarized that the human adenovirus serotypes that are being developed for HIV vaccines (Ad5, Ad26 and Ad35) are biologically different with respect to their sub-type, seroprevalence, cell-binding receptors, tropism, etc. He also provided evidence that they induce different gene-expression profiles and T-cell and antibody phenotypic functionality. In particular, Ad26 induces less profound and durable inflammation and interferon responses than Ad5 in vaccinated humans, but induces T-cell responses with a more functional memory phenotype than Ad5 in mice. These differences result in better protection with Ad26 vaccination against a SIVmac251 challenge in rhesus macaques. A DNA/Ad5 prime / boost regimen did not protect. However, Ad26/MVA and Ad35/Ad26 prime / boost regimens expressing the SIVsmE543 antigen did partially protect against heterologous challenge, and protection correlated with antibody titer. An Ad26/protein vaccine was also shown to partially protect against SIVmav251 challenge, as did Ad26/MVA followed by Ad35/MVA or Ad26/Ad35 against SHIV-SF162P3 challenge in rhesus monkeys. Finally, data to support the conclusion that administration of Ad26 does not result in increased activation status of total or vector-specific T-cells

in colorectal mucosa in a randomized, double-blinded, placebo-controlled clinical trial were presented.

Clinical experience on Ad26 and Ad35 (Dr. Jenny Hendriks, Crucell)

Crucell, now part of Johnson & Johnson, are evaluating the effectiveness of adenoviral vectors in heterologous prime / boost regimens in a number of indications including tuberculosis, malaria and HIV, Ebola and Marburg, and have developed a cell line based manufacturing system to enable low cost of goods for these candidates. It has previously been shown that a tuberculosis vaccine candidate Ad35-Ag85a/b (known as Ad35.TBS) boosts responses induced in BCG-primed adults with good cellular immune responses to the Ag85a/b antigen, and to date, nine Phase I studies and one Phase IIa have been completed with Ad35.TBS. An acceptable safety profile has been observed throughout, and in a Phase I infant study, Ad35.TBS was shown to induce a CD8 response at the highest dose ($1x10^{11}$vp) following 2 doses on days 0 and 56. A Phase IIb BCG prime/Ad35.TBS boost study (2 doses) in African infants is ongoing.

Crucell is also evaluating the Ad35 viral vector both alone and in combination with Ad26 for the ability of this regimen to elicit immune responses against the malarial circumsporozoite protein (CSP). Antibody responses were shown to be dose dependant with $1x10^{11}$vp giving the highest responses after 3 shots of Ad35.CSP at 0, 1 and 6 mo. At a shorter immunization regimen of 0, 1, and 4 mo antibodies, IFNγ and TNFα CD8 T-cells were induced at all dose levels tested. When Ad35.CSP was combined with Ad26.CSP in a prime boost regimen and subjected to a human sporozoite challenge, only one out of seventeen vaccinees was protected. Combining a prime of Ad35.CSP followed by RTS,S boost increased the cellular responses over RTS,S alone, in agreement with published NHP data. However this regimen did not result in increased protection in a human challenge study.

Two Phase I studies to evaluate immunogenicity of the HIV candidate Ad26.EnvA have been completed. In the first study to evaluate responses at 0, 1 and 6mo vs 0 and 6mo, the higher doses were shown to increase antibody breadth and epitope diversity and multiple cytokines were produced. In this study, Crucell demonstrated that in the presence of high levels of Ad26 vector-elicited NAbs, insert-specific Abs and T cells were boosted. A study to evaluate Ad35-Env/Ad26.EnvA (homologous and heterologous prime boost) at one dose at 0, 3mo and 0, 6mo has also been completed.

Novel Gorilla Adenovirus Vectors for Malaria Vaccine Development (Dr. Joe Bruder, GenVec)

GenVec are developing an alternative platform of adenovectors from adenoviruses that have low seroprevalence in the US population (6% of people have antibodies to the viruses). They have isolated adenoviruses from wild gorillas that are related to human type C adenoviruses; these are referred to as GC vectors. A single administration of GC vector expressing the murine malarial antigen *Py*CSP induces antigen-specific T-cell responses in mice that are significantly better than responses seen with Ad5 at doses of 10^7 and 10^8 vp. Better antibody responses are seen with the most promising GC candidate, GC46, than with Ad5 at the highest dose tested of 10^9vp. These immunological data supported progression of GC46 into a murine challenge study in the *P.yoelli* model where protection was higher than that seen with Ad5, although not significantly. GenVec is also developing GC46-based candidate vaccines for other indications including HSV and RSV. In these cases, these vectors have been shown to induce durable responses with a single dose. The RSV candidate has been shown to protect against murine RSV challenge.

GenVec, in partnership with the Naval Medical Research Center (NMRC) has selected GC46 for advancement into the clinic. GenVec plans to generate and manufacture, using a proprietary cell line to support manufacture of GC vectors, three malaria vaccine candidate GC46-*Pf* vectors: GC46-*Pf*CSP, GC46-*Pf*AMA1, and GC46-*Pf*TRAP. They are proposing to evaluate these vaccines in a human challenge study. Pre-IND discussions with the FDA are ongoing.

Clinical regulatory obstacles related to Adenovirus 5 (Dr. Tom Richie, US Naval Medical Research Center (NMRC))

Dr Richie reviewed the NMRC's experience with the development of an Ad5-based malaria vaccine candidate. The NMRC demonstrated the proof of principle that a DNA vaccine expressing CSP is able to offer protection against murine malaria challenge. Since that pivotal preclinical study in 1994, the NMRC evaluated a DNA vaccine combination vaccine (MuStDo 5) in a human malaria challenge study that included LSA3, TRAP, EXP1 and LSA1 as well as the cytokine GM-CSF to boost the immune response. However none of the subjects was protected. After demonstrating that a boost with Ad5 is able to protect rhesus macaques against challenge, the NMRC collaborated with Genvec to perform a human challenge study in individuals who received three shots of DNA-CSP + DNA-AMA1 followed by a Ad5-CSP + Ad5-AMA1 boost 24 weeks later (NMRC-M3V-D.Ad-PfCA Vaccine). Four out of fifteen subjects were sterilely protected. The protected individuals were found to have NAb titres below a threshold level, suggesting that pre-existing NAb to Ad5 many have interfered with protection. There appears to be a 'trade off' between higher ELISpot responses seen at the lower Ad5 (10^{10}vp) dose vs the higher antibody response seen at the higher Ad5 (10^{11}vp) dose. Total IFN-γ ELISpot and CD8+ T-cell responses to AMA1 were shown to correlate with protection in this study. Because safety concerns raised by the STEP trial preclude development of the heterologous DNA/Ad5 regimen as a product, further testing will be limited to experimental medicine studies in HIV low risk individuals. Questions to be addressed include adding a third antigen (TRAP) to CSP and AMA1 to increase protection, and investigating the cellular response mechanisms underlying sterile immunity.

Dr Richie touched on the potential safety concerns associated with Ad5, namely shedding which should not be applicable as Ad5 is non-replicating, persistent expression, integration within the host genome and characterization of the cell substrate to manufacture Ad5, i.e., origin, genetic manipulation, lineage, molecular characterization, infectious agents, genetic stability, tumorigenicity, oncogenicity, BSE, etc. Other hurdles may arise during Ad5 development, such as the need to evaluate and characterize the optimal dose regimen, i.e. dosage, 1 vs. 2 or more doses, regulatory route for heterologous prime / boost approaches, effect of pre-existing immunity to the vaccine vector, and interference between constructs or vaccines. As with all vaccines, scientific challenges remain, such as understanding the mechanism of disease and identification of a correlate of protection or protective epitopes to enable the induction of the appropriate immune response, and understanding whether a cell-mediated or antibody response, or both, is required.

Replicating Adenovirus serotype 4 Vaccine: H5N1 Influenza Phase I Results and Anthrax and HIV-1 Candidates (Dr Marc Gurwith, PaxVax)

Adenovirus 4 and adenovirus 7 are replication-competent viruses that have been licensed since the 1950s as vaccines for the US Military. As such they have an extensive safety record and have demonstrated convincing effectiveness in reducing acute respiratory disease among recruits. The platform is currently being evaluated by PaxVax for the development of vaccine candidates for additional indications, including seasonal and pandemic influenza, HIV and anthrax.

The pandemic influenza candidate Ad4-H5-VTN is currently in a Phase I study that is intended as a proof of concept study for the Ad4 platform, with evaluation of safety and monitoring of household contacts, as well as immunogenicity. The effect of pre-existing immunity to Ad4 is also being assessed. The regimen consists of three doses of up to 10^{11} vp of Ad4-H5-VTN on days 0, 56 and 114, with some patients receiving a protein boost in the form of a split vaccine administered 3-12 months after the last Ad4-H5-VTN immunization. The Ad4-H5-VTN vaccine appears to be safe with no dose-limiting toxicity observed, with no trend in adverse events increasing with dosage or number of doses. Ad4-H5-VTN was detected by PCR in rectal swabs of 80% of vaccinees at days 7 and/or 14 days post vaccination but there was no PCR-based evidence of transmission to household contacts. An almost 100% vaccine take was observed following three Ad4-H5-VTN vaccinations at the 10^{11}vp dose. However HAI titers were significantly increased by the protein boost, which is most likely related to the presence of pre-existing Ad4 titers which were shown to impact vaccine take. A Phase II study to evaluate the number of doses and optimal prime/boost interval of Ad4-H5-VTN is planned, and delivery of this candidate by alternative mucosal (tonsillar and intranasal) routes is ongoing. Phase I studies with the Ad4-HIV (2 vectors by oral and tonsillar administration) and Ad4-anthrax vectors are planned to commence in October 2013.

Ad4 and Ad7 vectors are replication competent in humans and can be delivered orally as enteric coated capsules or intranasally to induce mucosal immune responses. The vectors can be manufactured in the human diploid MRC-5 cell line. However, at least a 10-fold increased yield is obtained from A549 cells, a cell line derived from a human tumour; a Biologics Master File already exists for this cell line. In common with many novel tumorigenic cell lines, A549 offers many potential advantages such as low production cost and easy scalability, but faces developmental hurdles such as extensive characterization requirements from regulatory bodies and clear and consistent guidance on the route to licensure. Paxvax currently has active INDs for MRC-5 derived Ad4-H5 and Ad4-HIV candidates and A549-derived Ad4-anthrax candidates under review by the FDA. Early discussions with regulators concerning licensure of the Ad4 platform for broader indications and populations pertain to the need for assessment of environmental impact of the vector.

Status of Pox Virus Vector Development

Overview of Vaccine Research Centre perspective on key regulatory hurdles with adenovirus and pox vectors (Dr Barney Graham, Vaccine Research Centre, National Institutes of Health)

The NIH Vaccine Research Centre has completed 27 studies involving recombinant Ad products with a total of 4697 subjects. Analysis of all of the safety data from these subjects who received a dose of 10^{10}vp showed that most reported mild pain at the site of injection shortly after vaccination and some mild/moderate systemic reactogenicity in the form of headache, myalgia and tiredness that appeared to resolve after day 3. Transient and clinically insignificant but prolonged aPTT (activated partial thromboplastin time) signals have been seen in approximately 10% of subjects in the HIV and Ebola virus Ad5 and rAd35 studies, as well as in preclinical studies. aPTT is used to test for coagulation and to detect potential issues with blood clotting due to the presence of antiphospholipid antibodies. However, a false positive result can be detected where no clinical symptoms are present, and the test signal resolves within 3-4 weeks post vaccination. In light of this, Dr Graham questioned the utility and need for aPTT testing.

Dr Graham also argued to remove the requirement for routine ECGs and cardiac troponin assays from protocols evaluating modified vaccinia Ankara (MVA) in the clinic. These assays have been a

requirement in all US based trials involving MVA since 2004 as a result of myo/pericarditis that was observed during the US military smallpox vaccination campaign that utilised vaccinia virus (DryVax). However, MVA is a highly attenuated, non-replicating strain of vaccinia virus that has been established as a safer alternative to vaccinia virus and as a vector for recombinant vaccines that has been safely administered to over 120,000 people as part of a smallpox eradication campaign in the 1970s. In a publication reporting the safety surveillance of six Phase I trials of MVA vaccines, no participant had evidence of symptomatic or asymptomatic myo/pericarditis that meets the CDC-case definition and judged to be related to an MVA vaccine[2].

In common with other reports, the potency of Ad vectors varies depending on the immune response that is being measured. In a Listeria challenge model performed using low levels of candidate in order to detect a difference in potency, Ad5 was comparable to ChAd3 and both were more immunogenic than ChAd63 and Ad28. Ad35 was the least potent. The VRC rAd5 is a second generation rAd5 vector and contains an E3 and E4 deletion as well as deletion of E1 as compared to the first generation (Merck) vector. This results in reduced expression of adeno-specific proteins so that these vectors tend to direct the immune response to insert antigens rather than vector antigens. The VRC Ad5 vector was used in the HVTN 505 study, a Phase IIb HIV heterologous prime boost efficacy study performed in the USA in circumcised men with pre-existing Ad5 antibodies as an exclusion criteria; data to be published imminently in the New England Journal of Medicine.

HIV Canarypox Vectors in Phase III Clinical Trials (Dr. Danna Skea, Sanofi Pasteur)

The development of Sanofi Pasteur's ALVAC-based HIV vaccine program with three candidates: vCP205, vCP1452 and vCP1521, was reviewed. The vCP1521 candidate demonstrated 31% efficacy in the recent RV144 efficacy study in Thailand, in which it was used in a heterologous prime boost regimen with AIDSVAX B/E (VaxGen's recombinant protein gp120 adsorbed to Alum). vCP1521 is almost identical to vCP205, except that the *env*-coding sequence is derived from a TH023 strain (CRF01_AE is the predominant circulating recombinant form of HIV in Thailand) and the *env*, *gag* and *pro* gene sequences are inserted at position C6 rather than C3. The ALVAC-HIV (vCP1521) candidate, has now been administered to >8000 individuals, mostly as part of a heterologous prime / boost regimen with AIDSVAX B/E in HIV-uninfected adults. Forty-eight infants were also vaccinated in the HPTN 027 study.

The RV144 study was designed to administer ALVAC-HIV at 0 and 1 month, followed by a boost of ALVAC-HIV + AIDSVAX B/E at 3 and 6 mo. Subjects were tested for HIV-1 infection at Day 0, weeks 24 and 26, and every 6 months thereafter during the 3-year follow-up. Although 31.2% vaccine efficacy was detected at 3 years (p = 0.04) in the modified intent to treat (mITT) population, there was no significant difference in viral load among subjects who became HIV-infected during the study, between vaccine group and placebo group. Vaccine efficacy peaked at 60% at one year post the first immunization and coincided with peak level of vaccine immunogenicity.

In an effort to increase the responses seen with RV144 and accelerate the development of a globally effective HIV vaccine, Sanofi has joined the P5 'Pox Protein Public Private Partnership', which includes Novartis Vaccines and Diagnostics, HIV Vaccine Trials Network (HVTN), Division of Acquired Immunodeficiency Syndrome (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), United States Military HIV Research Program (USMHRP) and the Bill & Melinda Gates Foundation. The remit of the P5 group is two-fold: the licensure track is to pursue a strategy that will deliver the public health potential of this product as efficiently as possible, by substantiating and extending the ALVAC prime : protein boost concept to include higher risk populations in a broader geographic scope and to enhance the level of efficacy, prolong the

duration of protection and identify immune correlates of protection; and the research track is to optimise and validate the poxvirus prime: protein boost concept by evaluating next generation candidates and dosing regimens, for example by assessing other vector platforms, antigens and adjuvants. Three follow on clinical studies with vCP1521 are already underway or planned: one Phase II study includes boosting of RV144 vaccinated (HIV uninfected) individuals in Thailand to determine recall of immune responses induced in RV144, another phase II study in Thailand will enrol naïve (HIV uninfected) subjects and administer the RV144 vaccine regimen with an additional boost at 12 months, and one Phase Ib in South Africa will assess the immune responses to the RV144 regimen in HIV-uninfected South African adults.

ALVAC® is an enveloped virus derived from canary pox that is able to replicate in avian cells and therefore be manufactured using an avian continuous cell line. Replication in non-avian cells is blocked, which adds a safety feature. And because poxviruses replicate in the cytoplasm, issues associated with integration are minimized. The ALVAC® platform has been licensed for a number of veterinary vaccines including PUREVAX® to protect against feline rabies and feline leukemia virus (FeLV), RECOMBITEK® to protect against canine distemper and equine influenza and West Nile Virus (WNS).

University of Oxford's recombinant poxvirus programme (Dr Eleanor Berrie, The Jenner Institute, Oxford University)

The Jenner Institute is a research partnership between the University of Oxford and the Pirbright Institute. Its remit is to bring together investigators and resources to focus on translational research and to collaborate on design and development of human and veterinary vaccines. The intent is to maximise synergies across research groups and share essential core facilities and know-how, such as GMP facilities and access to disease challenge models. The Clinical Biomanufacturing Facility (CBF) is one of the core facilities at the Jenner offering expertise in adenovirus manufacturing and experience of process development, manufacture and release of various chimp and human adenovirus strains for various indications.

The CBF have been considering expansion into MVA manufacture. However, this brings a number of challenges such as onerous vector construction and generation of the viral seed as starting material using the homologous recombination technique, development of an aseptic downstream process, and inaccessibility of cell lines for manufacture and therefore reliance on a chicken embryo fibroblast process which has a low yield, and is difficult to produce at scale. The CBF is developing an alternative recombination strategy involving a bacterial artificial chromosome that expedites the cloning step, and results in a viral seed that is easier to characterise. The group are also investigating the use of cell lines rather than primary cells from embryonated eggs with the intent to perform bridging studies to demonstrate comparability between material derived from the different substrates. The MVA upstream process utilizes disposable, single use technology (SUT) and can be performed in a closed system that is robust and reliable. However, the downstream process is more challenging, as standard chromatography resins are not suitable for MVA and the process is difficult to validate as sterile connections are required throughout. The use of SUT within an academic environment is particularly challenging as funders typically support manufacture, but not the equipment required.

Development of recombinant poxvirus programmes can be accelerated by improved access to cell lines that enable continuous cell culture resulting in improved yields with lower cost of goods. Increased harmonization of requirements from the various NRAs on validation of sterile connection

systems, but also guidance on characterization of cell lines and viral vectors, as well as optimal infectivity assays would be advantageous.

Recombinant lentiviruses, arenavirus and measles virus (Dr Andrea Cara, Istituto Superiore di Sanita; Dr Lukas Flatz, Lausanne University Hospital; Dr Hussein Naim, Consultant)

Replication-incompetent vectors based on these three types of viruses are currently in preclinical development and are planned to move into the clinic within the next few years. Integrating, non-replicating lentiviral vectors have been successfully used in recent gene-therapy studies, and a single shot of a Lenti-ENV HIV vaccine candidate has been shown to be broadly immunogenic in a murine model. However integrating vectors are associated with safety concerns such as insertional mutagenesis/oncogenesis. A safer alternative involves the interruption of viral DNA insertion into host nuclear DNA by disrupting the integrase-reading frame of the vector. This restricts the expression of the vector to plasmids, resulting in antigen secretion or MHC-1 presentation of the gene product of interest. The level of transcription and therefore expression is lower than for integrating lentiviral vectors. However this approach is safer and still able to elicit broad and robust immune responses, including CD8+ induction, in murine and non-human primate models. These vectors, known as Integrase Defective Lentivirus Vectors (IDLV) are a promising new approach for various infectious diseases and cancer.

Arenaviruses are a negative stranded RNA virus family that infects mainly rodents, and include four species that can cause severe hemorrhagic zoonoses in humans. In contrast, lymphocytic choriomeningitis virus (LCMV) is a rare accidental human pathogen that causes aseptic meningitis that is mostly clinically unapparent. Recombinant LCMV has been engineered to be a replication-incompetent, non-integrating and apathogenic vector that has been shown to target dendritic cells, efficiently inducing long-lived antigen-specific T-cell responses following homologous prime / boost against various pathogens, including Listeria monocytogenes, vaccinia virus and vesicular stomatitis virus. In a heterologous prime boost regimen with Ad5, rLCMV expressing the HIV antigen gp 145 was shown to confer 62% protection against an SIV challenge in a NHP model, although there was no difference in the viral load as compared with the null control. These data, along with its low seroprevalence in humans, suggest that it be further evaluated as a vaccine vector for HIV-1 and other viruses. Hookipa BioTech AG, Vienna, Austria are evaluating the potential of the rLMVC vector in preclinical studies and are preparing for GMP production. Discussions with the FDA have been initiated and first in man clinical studies are planned for 2015.

The current status of recombinant measles virus vector approaches expressing additional transgenes or as a chimera expressing an alternative envelope protein was reviewed. The measles virus offers several attributes as a vector due to its proven safety and efficacy record in over a billion vaccinated people, its ability to induce long-lived protection after a single injection and the low cost of production. One potential drawback of the using the vaccine virus as a vector is the pre-immunity in almost all adults due to the MMR immunization campaign. However good humoral and cell-mediated immunogenicity has been induced after a single dose when boosting with MV as an aerosol or an alternative route to the prime measles vaccination. rMV expressing heterologous antigens are being developed for several indications including West Nile Virus, SARS, HIV and HPV.

Regulators' Perspectives

Dr Barbara Langer, Paul Ehrlich Institute; Dr Keith Peden, US Food and Drug Administration; Dr Richard Isbrucker, Health Canada

Representatives from the USA, Canada and the EU regulatory authorities reviewed the current regulatory requirements to assess viral vector based vaccines in the clinic, identified the available regulatory guidance documents, discussed lessons learned as well as highlighted areas where clear guidance does not currently exist.

Guiding principles for various aspects of vaccine evaluation have been provided in several WHO documents (refer to appendix for references). Although they provide a basis for setting national requirements for evaluation of cell substrates for production of vaccines, stability evaluation, non-clinical and clinical assessment of vaccines, the information regarding the viral vectored vaccines is limited to general considerations. Guidelines issued by FDA and EMA are fairly well harmonized with respect to characterization requirements, but regulators caution that these guidelines are also *generic*. In addition, ICH Guidelines for biotechnological products provide advice on cell-substrate derivation and characterization without any details regarding the viral vectored vaccines. Each viral vector based candidate is unique in terms of its attenuation mechanism, mechanism of action, production method, safety profile etc., and the safety, quality and manufacturing is considered on a case-by-case basis.

In considering the choice of viral vector for a particular indication, the regulators advise that the viral backbone should not be pathogenic. The developer must justify the selection of the particular vector for the indication with respect to its risk. Viruses with certain tropisms should be avoided such as neuro- or cardiotropic viruses and the potential interaction between the vaccine virus and other viruses need to be considered, such as potentially mobilizable cell-substrate viruses that could be present during manufacture or human viruses during clinical studies, for example HIV.

Replicating DNA viral vectors such as adenovirus (Ad4/Ad7) and a pox virus (vaccinia) have been licensed for several decades to offer protection against respiratory infections and smallpox respectively, however although effective, there are potential safety concerns associated with replicating vectors such as shedding and use in immunocompromised populations. Positive-strand or negative-strand replicating RNA viruses such as measles virus are considered safer, as they are limited to cytoplasmic replication removing the risk of integration (except for retroviruses), and their persistence is usually shorter than that of DNA viruses. Generally speaking, non- replicating viral vectors are considered to be safer and constitute the majority of candidates in clinical development, however replicating virus vectors continue to be evaluated as they may offer the immunogenicity and persistence required to protect against some intracellular pathogens for which other approaches have not been successful to date. Non-replicating DNA viruses include human adenoviruses (Ad5, Ad35, Ad26), chimpanzee adenovirus, gorilla adenovirus, poxviruses (NYVAC/MVA/ALVAC) and adeno-associated virus. Further developments include replicon, VLP or vesicular stomatitis virus-based vaccine candidates.

Some considerations and risks are common to all vectors and these need to be addressed as part of the regulatory package. For example pre-existing immunity to the vaccine virus may reduce the immune response in certain populations, or increase the safety risk as may have been the case with the STEP Ad5 study. Retention of efficacy after multiple vaccine doses, i.e., the development of immunity to vaccine vector needs to be evaluated and the dosing schedule optimized.

With respect to manufacture, viral vector based vaccines are produced by initial manufacture of the starting material known as a Master Viral Seed (MVS) which is prepared by passage of the recombinant vaccine virus in the production substrate. The MSV is then aliquoted and banked. A further viral seed bank known as the Working Viral Seed (WVS) is expanded and manufactured from the MVS, and the WVS is further expanded in the manufacture of the bulk vaccine. The production

cells are lysed (in the case of a non-lytic virus) and the virus is harvested and purified to produce the material for clinical studies.

The guidelines for characterization of the viral vector based vaccines have been harmonized (see Appendix for list) and require the following:

➢ Demonstration of stability of insert/transgene by PCR, expression, passage *in vitro* and/or *in vivo,* as well as stability of the attenuated phenotype, i.e. investigate the potential for reversion, recombination or replication in the vaccine recipient
➢ MVS is produced by passaging *in vitro*, or by biological recombination or reassortant; there is a need to document passage history of virus, raw materials used, and cell substrates
➢ Choose a well characterized cell substrate appropriate for the production of vaccine; where possible, those used for licensed vaccines, such as: SPF eggs, SPF-derived CEF, MRC-5, WI-38, or VERO should be used but novel substrates will be considered if they are more appropriate and sufficiently characterized.
➢ Tropism: evaluation of potential to infect non-anticipated organ or cell type, i.e., biodistribution study in rabbits or mice, as well as toxicity study in one of either rabbits, rats, or mice should be considered
➢ Increasingly it has become recommended to determine the full-length sequence of the vectored vaccine for Phase I clinical lots.
➢ *In vitro and in vivo* adventitious agent testing
➢ Dose and schedule in animals should justify dose and schedule in humans, and the route of inoculation may need to be considered. Additional studies may be warranted if the vaccine is to be tested in immunosuppressed individuals or an HIV-positive population.
➢ A Phase I clinical study will typically evaluate safety, tolerability, immunogenicity (response to antigen and vector) of escalating doses in healthy adults 18 to 45 years of age. Depending on the indication, transmission to close contacts and isolation of subjects may need to be considered.

Since a recombinant viral vector is considered a genetically modified organism, the EU has additional specific guidance documents with respect to evaluation of risk of viral shedding, inadvertent germline integration and requirements for environmental risk assessment. The purpose of this oversight is to identify and evaluate any potential risks for human health and the environment associated with clinical use of the product, to define measures to reduce any identified risk and to quantify the remaining risk.

Lessons learned:

➢ Aim to generate vaccine from a molecular clone, and characterize vaccine to demonstrate evidence of attenuation lack of pathogenicity. Follow good research and documentation practices for rescue, cloning and expansion of seed viruses.
➢ Use qualified cell substrates with known passage history from rescue onwards, and use qualified raw materials with Certificates of Analysis with special emphasis on animal-origin-free
➢ Choose a Contract Manufacturing Organization (CMO) that has experience in performing and scaling up viral vaccine GMP manufacture, and perform a technical and quality audit to confirm expertise, GMP compliance, capabilities and capacity. Execute a Quality Agreement and ensure frequent interactions and close oversight of the CMO.
➢ Allow sufficient time and resources to develop robust characterization assays and reagents to evaluate vector and cell substrate (infectivity, identity, expression, genetic stability, presence of adventitious agents, mycoplasma, general safety) and present a QC testing strategy to regulators during pre-IND and other consultations for advice.

➢ Ensure the GLP toxicology laboratory has been inspected by regulatory agencies for GLP compliance and that a technical and quality audit to confirm expertise, GLP compliance and capacity has been performed. Ensure frequent close oversight of the contractor, including on-site monitoring of critical activities such as formulation and dosing. Ensure studies are sufficiently powered and that they utilize the appropriate species.

➢ The selection of clinical investigators and sites is critical, and safety is paramount; established Good Clinical Practice including oversight and monitoring is essential along with clear stopping criteria to prevent or minimize injury. For some indications, it is not possible to demonstrate immediate clinical benefit, particularly without a correlate of protection, and consideration needs to be given as to how to generate preliminary efficacy data safely and efficiently beyond Phase I.

➢ Need to understand the impact of prior exposure to the vector, or to wild-type virus from which the vector is derived, in terms of safety, and the anticipated immune response.

➢ Investigators need to contemplate when and how shedding and transmission studies will be performed, if applicable.

Although several guidance documents do now exist, and an effort to harmonize across regulatory agencies continues, a particular NRA may have a different interpretation of the guidelines which could result in inconsistent expectations of regulatory requirements. Since each viral vector has some aspects that are unique, vaccine developers need to review the guidance documents and understand how they relate to their product and to anticipate the potential safety issues with respect to adventitious agents, stability, potential for reversion etc. All agencies encourage and advise early consultation to identify any potential hurdles.

Closing session and conclusions

Significant advances in the research and development of viral vector based vaccines have taken place over the last decade, resulting in a larger toolbox of potential vectors, cheaper and more reliable manufacturing methodology, improved understanding of the desired immunological mechanism and strategies to elicit the appropriate immune response. Along with these advancements has been an effort to co-ordinate and collaborate to develop guidance documents on several aspects that need to be considered for eventual licensure. Proof-of-concept efficacy studies in HIV and tuberculosis have been completed, albeit with sometimes disappointing results, but the field continues to advance with the conviction that viral vectors offer the best route to an effective vaccine for intracellular pathogens. The ability to induce high magnitude CD8+ T-cell responses through subunit vaccination has been achieved in the viral vector clinical trial arena, particularly using certain promising heterologous prime / boost approaches such as adenovirus-poxvirus heterologous prime / boost.

In the closing session of this meeting, the participants discussed remaining challenges, areas of future focus and identified issues where the WHO may be able to support in establishing current priorities and developing further guidance. There are three areas for further discussion with regulators, manufacturers and other relevant experts:

i) Development of novel vectors as potential platforms: this is driven by the need to overcome pre-existing immunity, particularly with the human adenoviral vectors as well as the desire to develop safer, more stable, highly immunogenic constructs that are able to accommodate multiple genes within a single product. There may be some resurgence in the development of replication-competent viruses as part of this effort, particularly for indications where the risk/benefit ratio of an effective vaccine may support regulatory approval.

ii)   Evaluation of the various viral vectors in heterologous prime boost regimens, which appears to be the most successful vaccination approach and may help to overcome anti-vector immunity and aid effective boosting of memory responses. However, licensure of a vaccine containing two products, most likely manufactured by different developers as a single regimen, is unprecedented. The assumption is that both products would need to be reviewed within the same package, but there are as yet no regulatory guidelines to support this approach and it is therefore perceived as a barrier. This has been identified as an important gap that needs to be addressed.

iii)   Improved manufacturing technology and a move towards continuous cell culture to produce virus at larger scale and with lower cost. Whilst this is a positive advancement, many cell lines are immortalised by dominant oncogenes, which may require extensive cell line characterization. This might limit the accessibility to the larger developers. A related issue is the use by some groups of manufacturing processes for products at the translational research stage, which may not be appropriate for licensure from a regulatory perspective. This raises several questions about the appropriate stage of advancement for such products or at least the need to pre-discuss and approve a bridging plan so that Phase 2b efficacy data can be used to support regulatory submissions for a product with a different manufacturing process.

The future of Ad5 development in the wake of the NIH meeting and the HIV efficacy study failures was debated. The Ad5 vector continues to show promise in many clinical studies for non-HIV indications and is a potent vector for boosting 6-24month old infants where there is very little or no pre-existing immunity, but has lost credibility in the broader vaccine field following the STEP study. The most recent analysis of the study data, and evaluation of the vector suggest that the safety issues were related specifically to the Merck HIV vector and the STEP/Phambili study design. There was consensus for the support for the continued evaluation of Ad5 in the clinic, particularly since the VRC HVTN 505 study incorporated Ad5 of a different backbone, expressing different antigens with no association with increased HIV incidence in vaccinated individuals. A potential concern with continued development of Ad5 for non-HIV indications is that investigators may be required to monitor patients for HIV status during the course of the study in order to rule out any potential association with the vector, which could be costly and onerous for late stage studies. Some members of the group suggested that non-Merck Ad5 based vectors could still be used for HIV providing that it is possible to demonstrate a meaningful biological difference between the two constructs, but the challenge will be in establishing how biological differences are defined in such a way that provides reassurance of safety. The group considered that it would take concerted and co-ordinated effort to work with regulators, funding agencies and other stakeholders to change the perception of risk associated with this vector, but that adenovirus vectors remain a key platform for vaccine development. It was suggested that the WHO could act as a broker to bring clarity in future forums on the Ad5 issue, and to endorse support for the platform in a non-HIV context. There appear to be compelling scientific reasons to continue with non-Ad5 adenovirus vectors for evaluation of HIV, malaria, tuberculosis and other vaccines.

It was proposed that the human vaccine field should collaborate more closely with the veterinary vaccine developers, who have licensed many viral-vectored vaccines for both commercial and companion animals. In spite of the many overlaps between the two disciplines, they have become distinctly separate with very little sharing of knowledge. The approach to better co-ordinate cross talk and scientific strategy is known as "One Health" or "One Medicine" and this is the topic of one of the current Grand Challenge Initiatives funded by Bill & Melinda Gates Foundation. Related to this paradigm is the notion that we should return to a focus on experimental medicine rather than product development for vaccines where correlates of protection do not exist. There was some

discussion as to whether the focus on target product profiles too early in development was curtailing development of potentially promising technologies because of non-ideal attributes that could ultimately be overcome in the event of clinical proof of concept. Once we have a better understanding of what type of immune response is required, we can use proven, less risky technologies to bridge.

There was general agreement that there is questionable scientific rationale to perform obligatory ECGs and troponin assays in clinical studies involving MVA since these vectors have a large and robust safety database. These assays are particularly in resource-constrained low and middle income countries where the risk benefit ratio for the candidate vaccines is very different to that in high income countries. There was broader discussion about how regulators should consider the attributes of a candidate if it is intended for a high mortality disease where the benefits of the vaccine would outweigh the perceived risk, and specifically to reduce the requirements for prior testing in high income countries, and increase the concomitant availability of data from low income country settings where the disease burden of a given pathogen is usually focussed. Acceleration of testing in local populations is more relevant and would significantly accelerate the development pathway to licensure for these critical vaccines, and potentially accelerate availability where the public health need is greatest. It was suggested that this is an area where the WHO may be able to intervene and support in establishing appropriate evaluation procedures for viral vectored vaccines. Further to this, there was discussion about regulatory pathways for vaccines targeting diseases that are predominant in low income countries, and whether WHO could do anything further to accelerate such regulatory pathways. A WHO representative indicated that EMA article 58 is seen as a very helpful regulatory pathway, as EMA can assess products with no intended European target population and such reviews are done jointly with WHO and WHO-appointed experts to provide the endemic country risk/benefit perspective. As one would expect, EMA performs regulatory reviews with the same rigour for article 58 applications as for marketing authorization applications.

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2.   Elizaga, M.L., et al., *Prospective surveillance for cardiac adverse events in healthy adults receiving modified vaccinia Ankara vaccines: a systematic review.* PLoS One, 2013. **8**(1): p. e54407.

Appendix

1. List of Guidance documents:

➢ ICH Topic Q 5 D: Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003280.pdf

➢ EMEA: Guideline on Quality, Non-clinical and Clinical Aspects of Live Recombinant Viral Vectored Vaccines
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/08/WC500095721.pdf

➢ EMEA: Guideline on clinical evaluation of new vaccines:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003875.pdf

➢ EMEA/CHMP/GTWP/367513/2006 On the development of a guideline on clinical monitoring and follow-up of patients exposed to gene therapy/gene transfer medicinal products:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003950.pdf

➢ EMEA: CPMP/SWP/465/95 Note for guidance on preclinical pharmacological and toxicological testing of vaccines:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004004.pdf

➢ EMEA/CHMP/VWP/164653/2005 Guideline on clinical evaluation of new vaccines:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003870.pdf

➢ WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. Replacement of Annex 1 of WHO Technical Report Series, No. 878:
http://who.int/biologicals/vaccines/TRS_978_Annex_3.pdf

➢ WHO Guidelines on Stability Evaluation of Vaccines:
http://who.int/biologicals/vaccines/Annex_3_WHO_TRS_962-3.pdf

➢ WHO Guidelines on Clinical Evaluation of Vaccines: regulatory expectations:
http://who.int/biologicals/vaccines/Annex_3_WHO_TRS_962-3.pdf

➢ WHO – Guidelines on Nonclinical Evaluation of Vaccines:
http://www.who.int/biologicals/publications/nonclinical_evaluation_vaccines_nov_2003.pdf

➢ FDA: Cell Lines Derived from Human Tumors for Vaccine Manufacture
http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM319573.pdf

➢ EMEA/CHMP/ICH/449035/2009 General principles to address virus and vector shedding
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002680.pdf

➢ EMEA/273974/2005 Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors
http://www.ema.europa.eu/docs/en_GB/document_library/Other/2009/10/WC500003983.pdf

➢ EMEA/CHMP/ICH/469991/2006 General principles to address the risk of inadvertent germline integration of gene therapy vectors
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002679.pdf

➢ EMEA/CHMP/BWP/473191/06 Procedural guidance on ERA for MPs containing GMOs
EMEA/CHMP/GTWP/125491/06 Guidance on scientific requirements for ERA of GTMPs containing GMO

Table 1: Overview of completed/ongoing clinical trials utilising ChAd vectors (Nicosia)

trial code (EudraCT number)	Phase	antigen and Ad vector	dose range (Ad)	dose range (MVA)	location	n.volunteers	volunteer cathegory	status
HCV001 (2007-004259-12)	I	HCV (NS3-5b) ChAd3, Ad6	$5x10^8$ to $7.5x10^{10}$ vp	n.a.	UK	41	healthy adults	completed
HCV002 (2008-006127-32)	I	HCV (NS3-5b) ChAd3, Ad6	$5x10^8$ to $2.5x10^{10}$ vp	n.a.	UK	32	HCV chronically infected patients (with or without concomitant IFN/RBV)	ongoing
HCV003 (2009-018260-10)	I	HCV (NS3-5b) ChAd3, MVA	$2.5x10^{10}$ vp	$2x10^8$ pfu	UK	20 / 11	healthy adults / HCV chronically infected patients (with or without concomitant IFN/RBV)	ongoing
HCV005	I/II	HCV (NS3-5b) ChAd3, MVA	$2.5x10^{10}$ vp	$2x10^8$ pfu	USA	175	Healthy intravenous drug users (IVDU)	ongoing
HIV-CORE002	I	HIVconsv ChAd63, MVA, DNA	$5x10^9$ to $5x10^{10}$ vp	$2x10^8$ pfu	UK	26	Healthy adults	completed
VAC033 (2006-005966-37)	I	Malaria (METRAP) ChAd63, MVA	$1x10^8$ to $1x10^{11}$ vp	$2x10^8$ pfu	UK	54	healthy adults	completed
MAL034 (2008-006804-46)	I/IIa	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$ vp	$2x10^8$ pfu	UK	43	healthy adults	completed
VAC036 (2007-004567-21)	I	Malaria (AMA1) ChAd63, MVA	$5x10^9$ to $5x10^{10}$ vp	$2.5x10^8$ pfu	UK	16	healthy adults	completed
VAC037 (2009-012591-27)	I/IIa	Malaria (MSP1) ChAd63, MVA	$5x10^9$ to $5x10^{10}$ vp	$5x10^8$ pfu	UK	16	healthy adults	completed
VAC038	I	Malaria (CS) ChAd63, MVA	$5x10^9$ to $5x10^{10}$	$2x10^8$ pfu	Ireland	24	healthy adults	completed
VAC039 (2010-018341-56)	I/IIa	Malaria (AMA1, MSP1, METRAP) ChAd63, MVA	$5x10^{10}$ vp	$1.25x10^8$ to $2x10^8$ pfu	UK	38	healthy adults	completed
VAC040	I	Malaria (METRAP) ChAd63, MVA	$1x10^{10}$ to $5x10^{10}$ vp	$2x10^8$ pfu	Kenya	30	healthy adults (semi-immune)	completed
VAC041	I	Malaria (METRAP) ChAd63, MVA	$1x10^{10}$ to $5x10^{10}$ vp	$1x10^8$ to $2x10^8$ pfu	Gambia	16 / 24	healthy adults (semi-immune) / children (2-6 years of age)	completed
VAC042	I	Malaria (METRAP) ChAd63, MVA	$1x10^{10}$ to $5x10^{10}$ vp	$1x10^8$ to $2x10^8$ pfu	Gambia	24 (5-12mo) / 24 (10 weeks)	Infants (2-12 months)	completed
VAC043 (2010-023824-26)	I	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$ vp	$2x10^8$ pfu	UK	42	healthy adults	ongoing
VAC044 (2010-024166-22)	I	Malaria (AMA1) ChAd63, MVA	$5x10^{10}$ vp	$1.25x10^8$ pfu	UK	30	healthy adults	ongoing
VAC045	I/IIa	Malaria (METRAP, CS) ChAd63, MVA	$5x10^{10}$ vp	$2x10^8$ pfu	UK	30	healthy adults	completed
VAC046	IIb	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$ vp	$2x10^8$ pfu	Kenya	60	healthy adults (semi-immune)	ongoing
VAC047	IIb	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$ vp	$2x10^8$ pfu	Senegal	60	healthy adults (semi-immune)	ongoing
VAC048	I	Malaria (METRAP) ChAd63, MVA, MatrixM adjuvant	$5x10^{10}$ vp	$2x10^8$ pfu	UK	22	healthy adults	ongoing
VAC050	I/IIb	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$ vp	$2x10^8$ pfu	Burkina Faso	380	Infants and children (2-17 months)	ongoing

Table 2: rAd vectors in development (Graham)

	Platform	Phase	Countries for Clinical Trials
Malaria	Ad35.CS (Crucell)	Phase 2 (human challenge trial)	USA
Malaria	Ad26.CS (Crucell)	Phase 2 (human challenge trial)	USA
Malaria	ChAd63 ME-TRAP	Phase 2b proof-of-concept efficacy	Kenya, Burkina Faso, Senegal
Malaria	ChAd63 CS, MSP1 and AMA1	Phase 2 (human challenge trial)	UK
Tuberculosis	Ad35 (Crucell/Aeras)	Phase 2 safety and immunogenicity	
Influenza	Ad4 (Paxvax)	Phase 1	
Hepatitis C	ChAd3, ChAd63 (Okairos)	Phase 1	
Ebola/Marburg	Ad35 & Ad26 (Crucell)	Pre-clinical only	
Ebola/Marburg	ChAd3 (Okairos)	Pre-clinical only	
Ebola	Ad5 (Crucell vector/VRC insert)	Phase 1	USA
Respiratory syncytial virus	PanAd (Okairos) GC-46 (VRC/GenVec)	Pre-clinical only	
Anthrax	Ad4 (Paxvax)		
HIV	Ad5 (Crucell and Merck)	Phase 2b	international
HIV	Ad5 (GenVec vector/ VRC inserts)	Phase 2b	International Phase 1; USA only Phase 2b
HIV	Ad35 (GenVec vector/ VRC insert)	Phase 1	USA
HIV	Ad26 (Crucell/Barouch)	Phase I	USA
HIV	Ad5/48 hexon loop chimera (Crucell/Barouch)	Phase I	USA
HIV	Ad35 (Crucell/IAVI)	Phase I	international
HIV	Ad4 (Paxvax)	Planned Phase 1	USA